

REMARKS

Entry of the foregoing and further and favorable consideration of the subject application is respectfully requested.

As correctly stated in the Official Action, Claims 24, 25, 27-30 and 35-43 are currently pending. Claims 24, 25, 27-30, and 35-43 stand rejected.

By the present amendment, Claims 24, 25, and 38-43 have been canceled, without prejudice to or disclaimer of the subject matter contained therein. Claims 27, 28, and 35 have been amended. New Claims 44-47 have been added. Support for new Claims 44-47 can be found, at least, in original claims 2, 11, 26, 29, and 30. No new matter has been added.

Request for Interview

Applicants submit herewith a Request for Interview to discuss any outstanding rejections. However, should the Examiner deem the present response to put the currently pending claims in condition for allowance, such an interview is, of course, unnecessary.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 24, 25, 27-30, and 35-43 stand rejected as allegedly indefinite. Claims 24, 25, and 38-43 have been canceled by the present amendment, thereby mooting this rejection as it applies to these claims.

Claims 24, 25, 27-30, and 35-37 stand rejected as allegedly unclear as to what is meant by an "otherwise" pathogenic bacteria and whether the "pathogenic microorganism

and the "otherwise pathogenic microorganism" are the same bacterium. Claims 24, 25, and 38-43 have been canceled by the present amendment, thereby mooting this rejection as it applies to these claims. Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, independent Claims 27 and 35 have been amended to delete "otherwise." Withdrawal of this rejection is respectfully requested.

Claims 24, 27, 35, and 38 are allegedly indefinite for the recitation of "is capable of." Claims 24 and 38 have been canceled by the present amendment, thereby mooting this rejection as it applies to this claim. Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, Claims 27 and 35 have been amended as suggested by the Examiner on page 3 of the Official Action. Withdrawal of this rejection is respectfully requested.

Claim 24 stands rejected as indefinite for the recitation of "a method for immunization, prophylaxis, or treatment of a vertebrate at risk or suffering from a disease caused by a pathogenic microorganism." The Examiner argues that it is unclear how a homologous antigen produced by a strain of *Brucella*, *Mycobacterium*, or *Vibrio* can induce protective or therapeutic immune response against any pathogen. The Examiner further asserts that the term "pathogenic microorganism" encompasses fungi, viruses, parasites, and bacteria from various species. Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, Claim 24 has been canceled, thereby mooting this rejection.

Claims 27 and 35 stand rejected as allegedly indefinite for being unclear as to how a homologous antigen produced by a strain of *Brucella* can protect against any pathogen. Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, Claims 27 and 35 have been amended to recite "*Brucella*" in place of "pathogenic micro-organism." Withdrawal of this rejection is respectfully requested.

Claim 38 stands rejected as allegedly indefinite for the recitation of "at least one gene" in connection with the recitation that the "at least one gene is a Cu/Zn SOD gene." Claim 38 has been canceled by the present amendment, thereby mooting this rejection.

The Examiner further alleges that Claim 38 is indefinite because it is unclear which microorganisms contain Cu/Zn SOD. Particularly, the Examiner questions whether *Vibrio* contains these genes. The Examiner suggests that Claim 38 should be limited to bacteria that were known to contain these genes at the time the presently claimed invention was made. Claim 38 has been canceled by the present amendment, thereby mooting this rejection.

The Examiner further argues that Claim 38 is indefinite as it is unclear how a homologous antigen produced by a strain of bacteria that has the Cu/Zn SOD gene can protect against any pathogen, including those which does not naturally contain this gene. Claim 38 has been canceled by the present amendment, thereby mooting this rejection.

Claim 40 stands allegedly rejected as reciting "wherein said at least one gene is one or both of a GroES and a GroEL gene." The Examiner asserts that this clause should be inserted into part (b) of the claim. This rejection appears to apply to Claim 41, not Claim 40. Claim 41 has been canceled by the present amendment, thereby mooting this rejection.

The Examiner further argues that Claim 40 (presumably Claim 41) is indefinite because it is unclear which microorganisms contain GroES or GroEL genes. The Examiner asserts that Claim 40 (presumably Claim 41) should be limited to bacteria that were known to contain these genes at the time the invention was made. Claim 41 has been canceled by the present amendment, thereby mooting this rejection.

Claim 40 (presumably Claim 41) also stands rejected as allegedly indefinite as unclear as to how a homologous antigen produced by a strain of bacteria that has the GroES or GroEL gene can protect against any pathogen, including those that do not naturally contain these genes. Claim 41 has been canceled by the present amendment, thereby mooting this rejection.

Claims 25, 39, and 42 stand rejected as allegedly indefinite for the recitation of "said attenuated or avirulent version of the pathogenic micro-organism." Claims 25, 39, and 42 have been canceled by the present amendment, thereby mooting this rejection.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 24, 25, 27-30, and 35-43 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabled. The Examiner acknowledges that the specification is enabling for a method for immunization, prophylaxis or treatment of a vertebrate at risk or suffering from Brucellosis comprising, *inter alia*, utilizing at least one of the genes for Cu/Zn SOD, GroES or GroEL to transform an attenuated or avirulent strain of *Brucella* to form a vaccine. However, the Examiner asserts that the specification does not provide enablement for a vertebrate at risk of or suffering from a disease caused by **any** pathogenic micro-organism comprising inserting at least one copy of **any** gene that induces a protective immune response. The Examiner also concludes that the specification does not enable the scope of 38 which "only limits the scope to at least one Cu/Zn SOD gene, but does not include which bacterium these genes come from or the pathogenic microorganism which is being treated." With regard to Claim 41, the Examiner asserts that the scope of this claim is only limited "to at least one GroES or GroEL gene, but does not include which bacterium these genes come from or the pathogenic microorganism which is being treated." The Examiner notes that the "recombinant vaccine art is highly unpredictable." Claims 24, 25, and 38-43 have been canceled by the present amendment, thereby mooting this rejection as it applies to these claims. This rejection, to the extent that it applies to Claims 27-30 and 35-37, as amended, is respectfully traversed.

On page 8 of the Official Action, the Examiner suggests that the "claims blanketly suggest that any gene from three completely different genus of bacteria can be used is not sufficient to enable the invention." Claims 27-30 and 35-37 are limited to the

immunization, treatment, or prophylaxis of a vertebrate at risk of or suffering from Brucellosis using an attenuated or avirulent strain of *Brucella* homologously overexpressing at least one antigen. Claims 27-30 are limited to particular species of *Brucella*. Claims 35-37 are limited to the use of *B. abortus* strain RB51 as the attenuated or avirulent strain used for immunization, prophylaxis, or treatment of Brucellosis. As noted above, the Examiner has admitted that the specification is enabling for such claim scope. Further, Claim 1 of U.S. Patent No. 6,149, 920, directed to a vaccine of similar scope and also examined by the present Examiner, was deemed to be enabled. Accordingly, Applicants respectfully submit that Claims 27-30 and 35-37 should **not** have been included in this rejection.

The Examiner states that "it would take undue experimentation for one to first locate a gene which when tested shows that it is capable of providing a protective immune response against disease in a pathogen." (Office Action, page 8). The Examiner argues that "the concept of extracting DNA from an organism and searching for a gene which may have the potential to encode an antigen ... is a vague intimation of a general idea that may or may not be workable." (Office Action, page 10). The Examiner appears to have misunderstood the presently claimed invention. This "searching" is not required, though it is also not prohibited, by the claims. While Applicants note that one skilled in the art is not bound to use currently known homologous antigens for *Brucella*, one skilled in the art can use such known homologous antigens such as the three exemplified in the specification. Steps a), b), and c) of the present claims require standard molecular biological techniques. Moreover, a number of homologous antigens are known for *Brucella*. One skilled in the

art can select one of these homologous antigens, *e.g.*, Cu/Zn SOD, GroES, GroEL, then homologously overexpress the gene via a multicopy plasmid in an attenuated or avirulent strain to achieve a more efficacious vaccine. In addition to the antigens described in the specification, other antigens can be used. Contrary to the Examiner's assertion, the presently claimed invention does not require that one of skill in the art "predict which genes out of the vast numbers of genes which encode antigens would be able to provide a protective immune response in a vertebrate." (Office Action, page 9).

The Examiner argues that Stevens et al. (*Comp. Immun. Microbiol. Infect. Dis.* 20(2):147-153 (1997)) disclose that GroEL proteins from *Bordatella pertussis*, *Legionella pneumophila* and *Mycobacterium bovis* fail to induce significant protection in mice or guinea pigs. Although the Stevens et al. publication is discussed in more detail below with regard to the obviousness rejection, Stevens et al., **incorrectly**, would not have predicted that the presently claimed invention would work and cannot be relied upon as dispositive of the enablement of the present claims. First, Stevens et al. do **not** use a vaccine **overexpressing a homologous antigen** as in the presently claimed invention. Stevens et al. merely inoculated test subjects with the **isolated** GroEL protein. Second, Stevens et al. also conclude that GroEL does not play an important role in conferring protective immunity to *Brucella*. (See Stevens et al., page 150). Accordingly, Stevens et al. might have predicted that attenuated *B. abortus* strains overexpressing GroEL would not be any more efficacious than a non-overexpressing *B. abortus* strain. Applicants have explicitly shown this to be incorrect. More importantly, it is difficult to see how Stevens et al. can demonstrate that the presently claimed invention is not enabled, when Stevens et al. has not

contemplated the nature of the presently claimed invention. Thus, Applicants respectfully submit that the disclosure of Stevens et al. should not be relied upon to determine whether the presently claimed invention is enabled.

Applicants respectfully submit that the presently claimed invention is enabled. The Examiner has not demonstrated why the presently claimed invention requires more than routine experimentation. Applicants respectfully submit that the testing involved in determining whether an antigen confers protective immunity is (and has been for some time) standard in the vaccine art and therefore does not require undue experimentation. Applicants are not required to demonstrate that every possible embodiment of the claimed invention works as intended. *See M.P.E.P. § 2164.08(b)*. Nor are Applicants required to carve out all possible embodiments that do not work. The test is whether one of skill in the art could determine whether an embodiment is operative or inoperative without expending more effort than that normally required in the art. *See Atlas Powder Co., v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984). While a working example is not required, it has been provided. *See Example 1*. The working example demonstrates the success of the presently claimed method. Moreover, Applicants submit that the claims are not unduly broad. Accordingly, withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 103(a)

Claims 24, 25, and 27 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Kontinen et al. (WO 94/19471) and Highlander et al. (U.S. Patent No. 6,180,112).

Kontinen et al. allegedly disclose a method and expression system for enhancing the secretion of hyperproduced homologous and heterologous exoproteins, including the use of multicopy plasmids for increasing gene expression. The Examiner argues that Highlander et al. disclose the use of whole cell vaccine compositions which overexpress a homologous leukotoxin antigen. The Examiner argues Highlander et al. disclose the use of heterologous antigens in addition to the overexpressed homologous antigens and the use of an attenuated strain of a gram-negative bacteria. Thus, the Examiner concludes that it would have been obvious to use multicopy plasmids to introduce homologous and heterologous antigens into attenuated or avirulent bacteria and administer the bacteria as a vaccine. Claims 24 and 25 have been canceled, thereby mooting this rejection as it applies to these claims. This rejection, to the extent that it applies to Claim 27, as amended, is respectfully traversed.

In order to establish a case of *prima facie* obviousness under 35 U.S.C. § 103, three basic criteria must be met: (1) there must be some suggestion or motivation to modify the reference or combine reference teachings, (2) there must be a reasonable expectation of success, and (3) the prior art reference(s) must teach or suggest all of the claim limitations. *See M.P.E.P. § 2142.* Applicants respectfully submit that the Examiner has not met these criteria.

Briefly, Claim 27 requires a) extracting DNA from the pathogenic micro-organism, b) obtaining a gene encoding an antigen that stimulates protective immunity, c) inserting the antigen gene into a multicopy plasmid, d) transforming an attenuated or avirulent strain of the pathogenic microorganism, and e) administering an effective amount of the vaccine

(attenuated or avirulent strain overexpressing the homologous antigen) to a vertebrate. Claim 27 further requires that the pathogenic micro-organism is one of four particular *Brucella* species. Applicants respectfully submit that each and every one of these elements of the presently claimed invention are not found in the Kontinen et al. or Highlander et al. publications, either alone or in combination.

With regard to Claim 27, the Examiner argued that Kontinen et al. disclose a method that could be used with:

any desired exoprotein, ... capsule, outer membrane and fimbria proteins from any Gram-negative bacteria, including *M. tuberculosis*, *Vibrio cholerae*. ... any protein toxins or secreted proteins from bacteria, surface proteins of any micro-organisms and antigen proteins or viruses may be overexpressed in the same manner as taught in the reference. Accordingly, this would include *Brucella* as recited in instant claim 27.

(Official Action mailed April 22, 2003, pages 11-12)(emphasis in original).

Applicants respectfully submit that this analysis by the Examiner is an obvious-to-try approach and does not provide the motivation required by 35 U.S.C. § 103. Kontinen et al. merely assert that their method could be broadly applied for generating large amounts of antigenic protein from any bacteria using the **gram-positive expression system**.

Applicants respectfully submit that this is insufficient to render obvious the selection of a particular genus of bacteria out of an extremely large number of possibilities for application in a **different** method, *i.e.*, that found in Claim 27.

As noted above, Kontinen et al. are using gram-positive bacteria for overexpression. That is, while Kontinen et al. suggest that antigenic proteins of *Mycobacterium* and *Vibro* might be used in their method, this would be **heterologous**

overexpression, not **homologous** overexpression achieved in the presently claimed invention. Applicants respectfully submit that this suggestion by Kontinen et al. is irrelevant to the presently claimed invention. The fact that Kontinen et al. blanketly suggest that any bacteria can be used in their heterologous system is not sufficient to render obvious the choice of a particular gram-negative bacteria in a homologous overexpression vaccine as in the presently claimed invention. Moreover, Kontinen et al. does not disclose or suggest that a homologously overexpressing pathogen makes a particularly efficacious vaccine.

Moreover, Claim 27 also limits the particular genus of *Brucella* to four particular species. The Examiner has not provided any concrete evidence of motivation in either the Kontinen et al. (other than a sweeping statement that "any bacteria" necessarily includes *Brucella*) or Highlander et al. publications that points the skilled artisan towards *Brucella*, or the particular species of *Brucella* claimed. Accordingly, neither the Kontinen et al. nor the Highlander et al. publications can render Claim 27 obvious.

Applicants submit that Highlander is **exclusively** directed to particular leukotoxin antigens of *P. haemolytica*. The Examiner cannot credibly extend the Highlander et al. publication to point towards any motivation to use the technique of homologous overexpression for *Brucella*. Further, Highlander et al. disclose two main uses of the leukotoxin antigens. Highlander et al. disclose the production of an inactive leukotoxin protein to be used as a vaccine itself. Col. 5, ll. 2-8. Highlander et al. also contemplate rendering a *P. haemolytica* strain avirulent or attenuated by the use of an inactivated leukotoxin or by negatively modulating activation of the active leukotoxin, not

homologously overexpressing an antigen in an already attenuated or avirulent strain. *See, e.g.*, Col. 4, ll. 48-54, Col. 8, l. 30 to Col. 9, l. 26. Highlander et al. specifically state, "in order to overproduce inactive leukotoxin, lktA transcription driven by a strong or inducible *P. haemolytica* promoter is desirable." Col. 43, ll. 4-8. Neither of these aims are relevant to the presently claimed invention. Highlander et al. also do not suggest or disclose that the use of homologous overexpression of toxin would lead to a more efficacious vaccine, as has been demonstrated in the presently claimed invention. There is no motivation in Highlander et al. to insert a gene for a homologous antigen in an attenuated or avirulent strain of bacteria, homologously overexpress the gene, and use the resulting recombinant strain as a vaccine with enhanced activity. If anything, Highlander et al. teach an entirely different method.

Applicants further point out that the Examiner's own arguments regarding the recombinant vaccine art undercut the Examiner's argument that the present invention is obvious. If the Examiner believes that the recombinant vaccine art is "highly unpredictable" (*See, e.g.*, page 8 of the Official Action), then there can be no "reasonable expectation of success" necessary for the presently claimed invention to be rendered obvious by the cited publications. These positions taken by the Examiner are incompatible. The lack of reasonable expectation of success is particularly glaring when one considers the breadth of the Kontinen et al. disclosure and the different purposes of the Kontinen et al and Highlander et al. disclosures in comparison to the presently claimed invention.

Applicants respectfully direct the Examiner's attention to the claims of U.S. Patent No. 6,149,920, the parent application of the present application, which was also examined

by the present Examiner. A copy of the '920 patent was submitted with the response filed February 5, 2003. Independent Claim 1 of the '920 patent is directed toward a vaccine, which the Examiner will observe could be produced and utilized according to the method of Claim 27, for immunization, prophylaxis, or treatment of Brucellosis. It is incomprehensible that the method for making and using a vaccine, which has already been deemed non-obvious by the Examiner, can be deemed obvious.

Finally, Applicants respectfully submit that the presently claimed invention is also non-obvious in light of the surprisingly efficacious results obtained. The specification and particularly Example 1 provides evidence that greater protection was achieved with the homologously overexpressing attenuated vaccine than with non-homologously overexpressing vaccines. These surprising results further point towards non-obviousness.

Applicants respectfully submit that the above comments demonstrate the non-obviousness of the presently claimed invention. The Examiner has not demonstrated that either the Kontinen et al. or the Highlander et al. publication, either alone or in combination, disclose or suggest each and every element of Claim 27. Additionally, the Examiner has pointed to no motivation, either in the two cited publications or elsewhere, that would lead one skilled in the art to the presently claimed invention. Accordingly, withdrawal of this rejection is respectfully requested.

Claims 24, 27, 28, 30, 35, 37, 41, and 43 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Kontinen et al. in view of Highlander et al. in further view of Stevens et al. (*Comp. Immun. Microbiol. Infect. Dis.* 20(2):147-153 (1997)). The

Examiner acknowledges that Kontinen et al. and Highlander et al. do not specifically disclose the use of *B. abortus* strain RB51. However, the Examiner argues that Stevens et al. disclose the vaccination of mice with attenuated *B. abortus* strain RB51 and the cloning of the GroEL gene into a regulatory plasmid vector to express GroEL at high levels. The Examiner argues that it would have been obvious to transform strain RB51 with GroEL in order to enhance the immune response. Claims 24, 41, and 43 have been canceled by the present amendment, thereby mooting this rejection as it applies to these claims. This rejection, to the extent that it applies to Claims 27, 28, 30, 35, and 37, as amended, is respectfully traversed.

Applicants initially note that the Applicants' comments above concerning the deficiencies of Kontinen et al. and Highlander et al. are equally applicable here. Stevens et al. do not disclose or suggest methods or means for achieving homologous overexpression. Stevens et al. take the gene encoding a *Brucella* antigen suspected to be a protective antigen (GroEL), subclone it into a plasmid, and place the plasmid in *E. coli*. The transfected *E. coli*. then expresses the *Brucella* GroEL gene through **heterologous** expression, a standard technique.

Stevens et al. then lyse the *E. coli* and purify the *Brucella* GroEL antigen from the lysate. The purified GroEL is **not** used to vaccinate any animals. It is used to test whether mice vaccinated with the *B. abortus* vaccine strain RB51 develop an immune response to the GroEL antigen, *i.e.*, via an *in vitro* immunoassay.

Stevens et al. do not overexpress any homologous antigen in the whole cell *Brucella* vaccine, do not suggest the possibility of overexpressing a homologous antigen in a whole

cell vaccine, do not describe or suggest any increase in protection against the pathogen, and did not intend to induce increased protection.

The Examiner has not indicated why one skilled in the art, in pursuing the goal of Stevens et al., *i.e.*, to prepare large amounts of protein to use in an *in vitro* immunoassay, would be motivated to use the bacteria from which the gene was initially isolated as Applicants have done. Stevens et al. use an *E. coli* expression system, which is the standard "workhorse" for performing such tasks. The objective of Stevens et al. and the expression system used by Stevens et al. are completely different from that of the present invention. Applicants respectfully submit that there is no motivation in either the Kontinen et al. or Highlander et al. publications that would have motivated one skilled in the art to modify the disclosure of Stevens to arrive at the presently claimed invention.

Moreover, as the present specification clearly demonstrates, the selection of *B. abortus* or other pathogen used for vaccination with homologous overexpression confers a selective advantage in the presently claimed invention. The present inventors have developed a vaccine having surprisingly increased efficacy by **homologously** overexpressing proteins such as GroEL in attenuated or avirulent pathogens. The present inventors have shown that this homologous overexpression system allows greater protection against pathogenic diseases than attenuated or avirulent vaccines expressing wild-type levels of proteins such as GroEL.

In contrast, Stevens et al. heterologously overexpressed the GroEL protein. The resulting protein was isolated and used in an assay, not as a vaccine. The heterologous expression system of Stevens et al. is simply a research tool to produce GroEL protein in a

conventional way to ascertain the importance (or lack thereof) of antibodies to GroEL in the immune response to brucellosis. Indeed, Stevens et al. conclude that the GroEL protein has minimal importance, if any role at all, in stimulating an immune response to *Brucellosis*. At page 150, Stevens et al. state that:

[N]either antibody or cell-mediated immune responses to GroEL appeared to be essential in conferring resistance to infection with strain 2308 in mice given the RB51 vaccine ... [the] antibody to GroEL does not appear to have an important role in providing immunity to brucellosis in cattle vaccinated with *B. abortus* strain ... [m]ost studies have shown that GroEL hsp proteins are ineffective as vaccines in preventing infections by various bacteria.

(emphasis added).

The only further study suggested by Stevens et al. (on page 152) is whether "vaccination of mice with the *B. abortus* GroEL protein will induce protective immunity, which is similar to that which occurs following vaccination of mice with attenuated strains of *B. abortus*." However, Stevens et al. discount the value of this experiment, stating, "[t]his would seem unlikely, however, because GroEL proteins from other bacteria have been ineffective as vaccines and as reported here immune responses to GroEL did not appear to be important in conferring protective immunity, which arose from vaccinating mice with *B. abortus* strain RB51."

As can be seen from these highlighted passages, Stevens et al. teach away from the present invention in concluding that the GroEL would not be an effective vaccine. Accordingly, there is no motivation to use GroEL as a vaccine *per se*, much less manipulate its expression so as to achieve overexpression in the pathogen from which the gene was derived. To the contrary, in light of the negative results of Stevens et al., one

skilled in the art would not see any value to **homologously** overexpressing the GroEL protein in *Brucella*. Teaching away is the antithesis of obviousness. There is nothing in the Stevens et al. publication that would motivate one skilled in the art to overexpress the homologous GroEL in *Brucella* to produce a vaccine. Contrary to the Examiner's suggestion that Stevens et al., in conjunction with Kontinen et al. and Highlander et al., renders the presently claimed invention obvious, the disclosure of Stevens et al. actually supports the non-obviousness of applicants' claimed subject matter.

Thus, none of the Kontinen et al., Highlander et al., or Stevens et al. publications, either alone or in combination render the presently claimed invention obvious. These three publications do not disclose or suggest each and every element of the presently claimed invention. To the contrary, Stevens et al. actually teaches away from the presently claimed invention. Thus, there is no motivation to combine these documents to arrive at the presently claimed invention as suggested by the Examiner. Moreover, the presently claimed invention produces surprising and unexpected results. Accordingly, withdrawal of this rejection is respectfully requested.

Conclusions

Applicants respectfully submit that Claims 27-30, 35-37, and 44-47 are in condition for allowance.

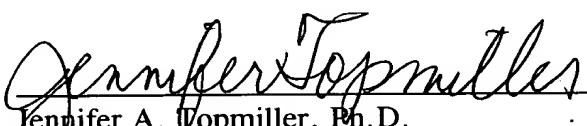
From the foregoing, further and favorable consideration of the subject application in the form of the issuance of a Notice of Allowance is believed to be in order.

If there are any questions concerning this amendment, or the application in general, the Examiner is respectfully requested to telephone Applicants' undersigned representative so the prosecution may be expedited.

Respectfully submitted,

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